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**Characterization of nivolumab associated skin reactions in patients with
metastatic non-small cell lung cancer**

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Abstract

Immune checkpoint inhibitors have led to considerable therapy improvement in cancer patients. Autoimmune side effects including skin reactions are frequently observed. In melanoma those include rash and vitiligo and were shown to be associated with a prolonged overall survival. Little is known about skin reactions in NSCLC patients during immunotherapy.

Here, we retrospectively investigated immune-related adverse skin reactions (irAEs) in 40 patients with metastatic non-small cell lung cancer (NSCLC) treated with the anti PD-1 antibody nivolumab. 7 out of 40 patients (17%) developed an irAEs.

Skin irAEs correlated with tumor responses in 5 of 12 responders (42%) as compared to 2 of 27 non-responders (7%). Histologically, scaly plaques showed dermatitis consisting mainly of lymphocytes.

We observed a positive correlation between skin irAEs and tumor responses in patients with NSCLC treated with nivolumab. Patterns of lymphocytic skin infiltration differed depending on the histological tumor subtype (adenocarcinoma versus squamous cell carcinoma NSCLC).

Introduction

Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers and can be divided into two major histological subtypes: adenocarcinoma (AC) and squamous cell carcinoma (SCC). Standard first-line treatment of metastatic disease consists of platinum-based chemotherapy. In selected molecular-defined subgroups the first-line therapy is a targeted therapy using tyrosine kinase inhibitors (TKI).¹

The introduction of immunotherapy has significantly improved therapy outcome. Two phase III trials with the anti-PD1 (anti-programmed death 1 receptor) antibody nivolumab led to its approval in 2015 after it had been approved for metastatic melanoma in 2014.²⁻⁵

PD1-checkpoint inhibitors block the interaction between PD1-receptors on T-cells and their ligand PD-L1. Blocking PD1-receptors enhances T-cell response against cancer cells.⁶ In turn, activated T-cells can cause autoimmune-mediated side effects, such as skin rash, colitis, hepatitis or pneumonitis. These are generally manageable and reversible in most cases. Rash and pruritus are frequent immune-related dermatologic adverse reactions (skin irAEs) during immunotherapy, which have been reported to occur in up to 25 % of melanoma patients.^{4, 5, 7, 8} Toxicity data for anti-PD1 approval in NSCLC reports skin irAEs in approximately 10% of patients^{2, 3, 9}, but as of recently higher rates have emerged.^{10, 11} Grading of toxicity is commonly classified according to Common Terminology Criteria for Adverse Events (CTCAE version 4.0). Management of skin irAEs includes symptomatic treatment with antihistamines and topical steroids, but may be complemented with systemic immunosuppression in severe cases.¹² Previous data strongly suggest that skin irAEs under anti-PD1 therapy are associated with increased overall survival (OS) and may serve as a parameter to predict a better therapy response.^{13, 14}

The aim of this study is to i) characterize skin irAEs in patients with advanced lung cancer treated with nivolumab and ii) to specify the pattern of T-cell infiltration in biopsies of these skin lesions.

Materials and Methods

Assessing tumor response to therapy with nivolumab and skin irAEs

After approval by the local ethics committee (EKSG Nr. 16/059) and in accordance with the Declaration of Helsinki Principles, we retrospectively analyzed patients with metastatic NSCLC treated with nivolumab (Opdivo®, Bristol-Meyers Squibb, SA). Treatment was initiated between January 2015 and February 2016 within an early access program at the Cantonal Hospital St. Gallen (Switzerland). Patients had at least one treatment cycle with the standard dose of 3 mg/kg i.v. over 60 minutes every two weeks. Tumor response was evaluated using computed tomography (CT) and was categorized as progressive disease (PD), stable disease (SD) and partial remission (PR) according to RECIST criteria 1.1.¹⁶ We further assessed results of skin examinations that had been performed during patient visits and classified skin irAEs according to clinical dermatological criteria.

Immunohistochemistry analysis

Histology of punch biopsies from untreated skin rash was available from 4 patients, of which 2 had SCC and 2 had AC. Biopsies of the SCC patients were taken from the right forearm and right lower limb and of the AC patients from the right cheek and left lower limb (one each, respectively). All punch biopsies had been performed by a dermatologist. Histopathological analyses were conducted independently by a pathologist and a dermatopathologist. From each representative paraffin-embedded

skin block four microns-thick sections were obtained for HE and immunohistochemistry (IHC) staining (CD3, CD4 and CD8). For each sample we determined lymphocyte counts in the epidermis and dermis per 1mm² (= 4 random high power fields).

Results

Tumor response and skin irAEs in NSCLC under nivolumab therapy

We identified 41 patients treated with at least one single dose of nivolumab. One patient was excluded because consent was refused. Patient age ranged from 46 to 88 years (mean 65 years). 22 (55%) were male and 18 (45%) female (Table 1). On average, the 40 patients were treated with 7 cycles, ranging from 1 to 25 cycles. The interval between treatment initiation and appearance of a skin irAE was 3 cycles.

23 (57%) of our patients had ACs, 14 (35%) SCCs and in 3 (8%) the tumor displayed features of both subtypes (mixed subtype). 27 (67%) showed disease progression under nivolumab therapy. We were unable to assess disease progression in one individual because the patient had died due to ileus before a re-staging could be performed.

7 (17%) developed a skin irAE under treatment. All skin reactions were classified grade I or II (CTCAE 4.0). In this subpopulation 4 (57%) were scaly plaques (Figure 1A/B) and 3 (43%) intense pruritus without visible skin lesions. Only 2 (29%) with skin irAEs had PD, as opposed to all patients, where the majority had PD. Furthermore, skin irAEs correlated with tumor responses in 5 of 12 responders (42%) as compared to 2 of 27 non-responders (7%).

Immune infiltrate patterns of skin rash under nivolumab therapy correlates with NSCLC subtype

Immunohistochemistry of the skin biopsies showed that the distribution of CD3+ T-cells in the inflammatory skin infiltrate was dependent on the cancer subtype (Figure 1F/G). We observed an analogous cell distribution pattern among cytotoxic CD8+ T-cells. In patients with SCCs the lymphocyte infiltrates were more prominent above the basal cell membrane whereas patients with ACs showed CD8+ T-cell infiltrates more accentuated towards the dermis (Figure 2A-D). Quantification of the T-cell infiltrates further supported this observation: the ratios of CD8+ T-cells found in the epidermis compared to all CD8+ T-cells of two patients with SCCs were 22% and 44%, respectively, while in two patients with ACs the ratios were 3% and 10%, respectively (Figure 2A-D).

Discussion

Autoimmune-mediated side effects affecting different organs are known to occur during treatment with immunotherapy. In NSCLC patients treated with nivolumab skin eruptions and pruritus have been reported in approximately 10% or higher.^{2, 3, 9-11} This was also reflected by our study. Several authors reported higher numbers of skin irAEs in melanoma patients under nivolumab treatment.^{4, 5, 7, 8} An association between appearance of skin irAEs and overall survival in melanoma has been reported.^{13-15, 17} Similarly, our data suggests an association between skin irAEs and tumor response. The histological analysis of the rashes on the one hand allowed us to rule out clinical differential diagnoses of eczema and psoriasis and on the other hand revealed that the rashes were characterized by an inflammation rich in CD3+ T-lymphocytes. Immunophenotypisation showed analogous lichenoid

patterns among rashes with SCC-patients. They displayed distinct epidermotropic inflammatory infiltrates consisting mainly of cytotoxic CD8+ T-cells. Civatte bodies (damaged basal keratinocytes) suggest that these lymphocytes induce keratinocyte death. From these findings we deduct that the skin of these patients harbored autoimmune T-cells that were activated and attacked healthy keratinocytes. It is further possible that tumor-specific T-cells, activated by anti-PD1 treatment, migrated to the skin. Since T-cells can migrate and scan antigens presented by MHC-I molecules on all body cells, they can recognize the same antigen at any body site. It is possible that keratinocytes express antigens that are identical or very similar to those of the tumor, known as antigen sharing or as molecular mimicry, respectively.¹⁸ In our point of view, antigen sharing can presently be considered the most likely cause, as lung SCCs and keratinocytes both produce similar proteins including several cytokeratins.¹⁹ In contrast, the histology of skin rashes of AC patients showed inflammation with lymphocytes located predominantly below the basal cell membrane within the dermis, which contains glandular structures (e.g. eccrine sweat glands). Those may share antigens with lung ACs.

Limitation of the study was the small sample size of 40 patients. One needs to keep in mind that immunotherapy for treating NLCSCs has only recently been approved and was provided through the early access program. We are confident that the recent approval now enables broader access to immunotherapy, which will allow our findings to be compared to future investigations from larger sample sizes.

In conclusion, our results suggest that the tissue tropism of lymphocytes in skin irAEs may be more specific than previously known, opening new opportunities for elucidating the underlying molecular mechanisms.

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Figure legends:

Figure 1. a. Skin rash under nivolumab in a patient with squamous cell carcinoma (SCC) of the lung or **b.** adenocarcinoma (AC) of the lung. **c.** Histopathology shows an inflammatory infiltrate migrating into the epidermis in SCC patients (hematoxylin eosin (HE), 100x). **d.** Apoptotic keratinocytes, also called Civatte bodies, are visible in SCC patients (HE, 200x). **e.** The infiltrate does not seem to migrate in AC patients (HE, 100x). **f.** Immunohistochemistry shows that the inflammation consists mainly of lymphocytes in both SCC (CD3, 100x) and **g.** AC (CD3, 100x) patients. The basal cell membrane is outlined in red.

Figure 2. a.b. Immunohistochemistry with a CD8 stain of skin rash of 2 SCC patients (CD8, 100x) and **c.d.** 2 AC patients (CD8, 100x). The migration of the inflammation appears to differ among tumor subtypes. The number in the upper right corner of each panel indicates the percentage of CD8+ T-cells in the epidermis compared to the total of CD8+ T-cells of 4 high-power fields. They illustrate that the relative CD8+ T-cell count in the epidermis is greater among 2 SCC patients than among 2 AC patients.

322 **Table 1.** Patient characteristics.

Parameter		
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Patient number		40
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Age (years)	Mean	65.5
	Range	46 - 88
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Gender	Male	22
	Female	18
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Histological type	Adenocarcinoma	23
	Squamous cell carcinoma	14
	Mixed type	3
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Nivolumab cycles	Mean	7
	Range	1 - 25
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Nivolumab cycles until adverse skin reaction	Mean	3
	Range	2 - 8
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Type of adverse skin reaction	Plaques	4
	Pruritus	3
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Tumor response in patients with adverse skin reaction	Response	5
	No response	2
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Table 2. Clinical presentation and histology of 4 patients

Patient	Gender, age in years	Onset (after cycles/days)	NSCLC ^a	Clinical presentation	Histology
1	m; 69	2/15	SCC ^b	Focal plaques with thick squamae on the extremities, with burning sensation, no mucosal involvement.	Lichenoid dermatitis with interface-component at the dermo-epidermal junction and CD8+ cell infiltrate in the epidermis and dermis.
2	m; 68	2/37	SCC	Focal plaques with thick squamae on the extremities, mild pruritus and burning, no mucosal involvement.	Lichenoid dermatitis with interface-component at the dermo-epidermal junction. CD8+ cell infiltrate visible in the epidermis, with apoptotic keratinocytes, and the dermis.
3	f; 86	1/7	AC ^c	Focal scaly plaques on the extremities with pruritus, no mucosal involvement.	Spongiotic dermatitis with accumulation of CD8+ T-cells below the basal cell membrane, sparse affection of epidermis.
4	m; 59	8/111	AC	Focal erythematous plaques with fine scaling on the upper face, no mucosal involvement.	Lymphocytic infiltrate with accentuation in the deep dermis. Sparse interface component with sporadic vacuolization of basal keratinocytes.

^aNSCLC: non-small cell lung cancer

^bAC: adenocarcinoma

^cSCC: squamous cell carcinoma